NudiKey: an illustrated, interactive identification key to the families of Australian heterobranch sea-slugs (Mollusca: *Gastropoda*)

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ABSTRACT

The Australian heterobranch sea slugs are popular among the public and are also important as potential indicators of environmental change. Despite this, there is no simple key for their identification and, as a result, most attempts at identification are made by comparative reference to books, websites, social-media or through museums and correspondence with taxonomists. At present there is no opportunity for users to self-interact with a key online. Therefore, development of an interactive taxonomic key that allows users to identify a sea slug specimen without any previous taxonomic knowledge fulfills a practical need by providing a valuable identification tool for amateurs and the general public. The objective of NudiKey is to summarise the essential diagnostic characteristics of the epibenthic Australian heterobranch families and, using the freely available Lucid v3.3 software and high quality photographs of living animals, develop a well-illustrated, user-friendly, interactive key.

Key words: Nudibranch, Sacoglossan, Pleurobranch, opisthobranch, Lucid

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Introduction

Heterobranch sea slugs, many of which were formerly known as opisthobranchs (Schrödl *et al.* 2011) are highly diverse, with over 6900 known species worldwide (Rosenberg 2014). More than 3400 species occur in the tropical Indo-Pacific, which forms the geographic centre of diversity for this group (Gosliner *et al.* 2015). On the Australian east coast, their diversity is highest in the tropics, with more than 1000 species (Rudman and Willan 1998) reported from the northern Great Barrier Reef. This diversity attenuates with latitude to 364 species in the temperate areas of Victoria and northern Tasmania (Burn 2006).

Sea slugs are popular with recreational divers, underwater photographers and the general public (Thompson 1976b; Behrens *et al.* 2005; Cobb and Willan 2006; Nimbs *et al.* 2015). Not only do they have popular appeal but they also hold scientific importance (Nimbs *et.al.* 2016): several species are considered invasive (Willan 1987, 2004; Hewitt 2002; Sliwa *et al.* 2009; Giacobbe and De Matteo 2013) and many can be useful indicators of broader biodiversity (Smith 2005). Their popularity has made them an ideal group for citizen science monitoring projects such as the *Sea Slug Census* (Smith and Davis 2013; Nimbs *et al.* 2016), however their high species diversity means that accurate identification generally requires a level of taxonomic expertise.

In spite of their popularity, a cost-free, broad-platform, interactive key is not presently available to identify this conspicuous gastropod subclass. As a result, identifications may rely on access to traditional dichotomous keys scattered among the primary literature or by comparative reference to identification guides (Willan and Coleman 1984; Wells and Bryce 1993; Marshall and Willan 1999; Cobb and Willan 2006; Coleman 2015; Gosliner *et al.* 2015), websites (Cobb and Mullins 2003), mobile applications based on simple diagnostics (Cobb 2016), social media (NudiBase 2014), websites (Rudman 1999) or through correspondence with museums, taxonomists and keen amateurs (Cobb and Mullins 2003).

Until the advent of interactive software, dichotomous keys continued to be the backbone of taxonomic identification systems in spite of their deficiencies (Dallwitz *et al.* 2000). A major limitation of dichotomous keys is that they fail to offer users options in terms of the sequence of paired character states. Thus, should a user be unable to answer a question, an identification often cannot be reached (Dallwitz *et al.* 2000). Moreover, where a user reaches a particular end point, and the identification proves to be incorrect, finding where things went wrong involves considerable backtracking. To overcome these deficiencies some level of specialised knowledge is often required and frequently results in the outsourcing of diagnostic skills (Norton *et al.* 2012).

Modern, interactive keys have a number of advantages over traditional dichotomous keys: characters used to identify taxa are not numerically limited and their value/s or order may be altered during the identification process; the presence of an error, by either the user or the author, may not necessarily preclude correct identification; and users may articulate uncertainty by entering a range or more than one value for a characteristic (Dallwitz *et al.* 2000). These computer-based keys are often augmented with illustrations which can include photographs of key features. Ready availability via the internet or portable media has helped overcome the pragmatic deficiencies of dichotomous keys by providing a means of transferring taxonomic expertise to non-specialists.

In response to the need for a key to the Australian sea slug families, NudiKey was developed to consolidate taxonomic data into a single matrix based, multi-entry key. The key was designed to be the first (in a series of electronic keys to be developed later) aimed at resolving identification of adult Australian sea slugs to species level. Taxonomy and nomenclature follow that published online by WoRMS (2015) at the time of writing (November 2016).

Materials and Methods

Intended for use by amateurs and non-scientists, the key scope was limited to those families listed in regional checklists and field guides such as Burn (2006, 2015); Cobb and Willan (2006); Coleman (2008, 2015); Debelius and Kuiter (2007); Gosliner *et al.* (2008, 2015); Wells and Bryce (1993); Willan and Coleman (1984); Yonow (2008). Most of the predominantly pelagic and infaunal families are not treated in detail among popular literature and they tend not to be the focus of amateurs, divers, photographers and non-scientists. Thus, with the exception of Fionidae and Glaucidae, these families are not included in the key. External morphological characteristics diagnostic to family were summarised from the existing literature, particularly Rudman and Willan (1998) which, although now somewhat superseded with regard to higher taxonomy nevertheless provided a useable synthesis of most characteristics at the familial level.

Qualitative characters (in the key termed 'features' and 'states') were defined for all epibenthic Australian families ('entities') and arranged into anatomical categories. Each feature and its relevant range of selectable states were illustrated using photos of living animals. Features, states and entities were input into LucID3 Builder version 3.3.3 (available from http://www.lucidcentral.com/en-us/software/lucid3.aspx).

The Lucid builder software incorporates a 'score analyser' tool which quantifies the differences among feature states used to distinguish entities and illustrates any potential weaknesses in the key. This tool was used to determine whether there were sufficient differences scored among the entities to unambiguously resolve most identifications. The Lucid Builder allows the attachment of media to both feature and entity entries as either hypertext markup language (HTML) or image files. A separate HTML based information page was developed for each feature and entity using KompoZer version 0.7.10 (20070831) what-you-see-is-what-you-get (WYSIWYG) software available from http://www.kompozer.net/. Production using HTML was selected as it allows a greater degree of flexibility at the development stage, pages can easily be converted to PDF as stand-alone documents and they are relatively easy to modify using WYSIWYG software. Each information page was attached as a pop-up link to its particular feature or entity.

Each of the selectable states related to each feature was described in detail within its pop-up information page and was illustrated using cropped images overlaid with graphics (where required) to highlight the particular state. Basic image manipulation such as cropping and graphic overlay were processed using Microsoft Powerpoint. In a few cases, video clips of behavioural features were included and these were filmed using an Olympus TG-3 compact camera in a PT-056 waterproof housing. The clips were edited using Microsoft Movie Maker and uploaded as MP4 files. In most cases, photographs were sourced from the author's library or from other malacologists. There were instances, however, where a photograph of particular feature was required, for example the gill leaflets in Phyllidiidae, and photographs of these were taken during field activities associated with other sea slug research (Nimbs and Smith 2016).

In anticipation that most of the intended users will require additional information on the operation and development of NudiKey, supporting information was developed and, using HTML, included as a set of 'front-end' web pages. These pages include: how to use, scope and limitations, glossary, acknowledgements, photo credits, references and system requirements and troubleshooting. Information used to develop the system requirements and how to use pages were derived from documents developed by LucidCentral (2016).

Results and Discussion

The first version, NudiKey v1.0 is available at http://keys.lucidcentral.org/keys/v3/NudiKey/.

A total of 16 features are defined (for 63 entities) and arranged into anatomical categories: head (7 features), body (5), gills (3) and shell (1) (Table 1). In the key interface, these categories are arranged into a series of nested collapsible lists which may be expanded to list the states relevant to the selected feature. All feature choices are scored as multistate with a total of 84 states available for selection. None of the states are dependent and thus all options in the key remain unrestrained.

Table 1. Sea slug features and states listed in NudiKey.

Category/feature/state
Head
Head shield
1. Head shield has lobes that extend over the front and sides of the shell, lower lobes have a deep cleft
2. Head shield is triangular, lobed and the rear portion narrows to cover the front of the visceral hump
3. Head shield is triangular and the rear lobe is erect
4. Head shield is triangular and a siphon projects from behind, occasionally arching forward
5. Head shield has two frontal lobes with recurved edges that fold upwards
6. Head shield has two lower lobes folded down and under the head
7. Head shield side lobes appear as rolled, siphon like tentacles and the rear, triangular lobes cover the front of the shell
8. Head shield is small and there is no obvious division from the rest of the body
9. Head shield rear lobes may resemble rhinophores
Oral Veil
1. Oral veil is tuberculate/papillate
2. Oral veil forms lateral tentaculate lobes
3. Oral veil is reduced to form palmate tentacles
4. Oral veil is wide, appears to be fused with the mantle
5. Oral veil is bilobed
6. Oral veil forms a hood with papillate margins
Eye spots
1. Eye spots are posterior to the rhinophores
2. Eye spots are anterior to the rhinophores
3. Eye spots are at the base of the rhinophores
4. Eye spots are close together on the top of the head
5. Eye spots are between the head shield lobes
6. Eye spots are between the rhinophores or cephalic tentacles
Oral tentacles
1. Oral tentacles are rolled
2. Oral tentacles are palmate
3. Oral tentacles are auriculate
4. Oral tentacles form lobes or flaps (lappets)
5. Oral tentacles are short
6. Oral tentacles are long and tapering
Rhinophore protection
1. Rhinophores are retractile
2. Rhinophores are contractile
Rhinophore sheaths
1. Rhinophore sheaths are low, simple and smooth
2. Rhinophore sheath openings are tuberculose or papillose
3. Rhinophore sheath openings are flared or scalloped
4. Rhinophore sheaths are highly modified and resemble dorsal cerata
Rhinophore shape
Rolled
1. Rhinophores are rolled with auriculate flaps
2. Rhinophores are rolled and do not have auriculate flaps
3. Rhinophores are rolled and widely-spaced
Rod-like
1. Rhinophores are smooth and rod-like, lack papillae, annulae and lamellae and often taper

2. Rhinophores have, small, rounded or occasionally hair-like papillae
3. Rhinophores are annulate
4. Rhinophores have densely-packed lamellae
5. Rhinophores are bifid

Clubbed

1. Rhinophore clubs have horizontal and/or diagonal lamellae
2. Rhinophore clubs have vertical lamellae

Rhinophore tips

1. Rhinophores have a terminal knob
2. Rhinophores have a terminal clavus
3. Rhinophore tips are palmate/papillate

Body

Body Shape

1. Body is elongate, smooth and cylindrical
2. Body is plump, round or ovate
3. Body is narrow or elongate often with multiple processes
4. Body is bulky with a distinct head
5. Body is elongate, sometimes tapering, with smooth or ruffled margins
6. A shell is present

Mantle skirt

1. Mantle skirt is reduced forming a ridge along the sides of the body
2. Mantle skirt is highly reduced forming a rounded frontal ridge or veil
3. Mantle skirt is large and may completely cover the foot
4. Mantle skirt is slightly reduced with wide margins and longitudinal ridges
5. Mantle skirt has undulate or sinuous margins
6. Mantle skirt is reduced forming thickened lobes

Visceral hump

A visceral hump is present

Siphon

1. Exhalant siphon is present at the rear of the shell
2. An erect siphon is present posterior to the head shield
3. Exhalant siphon is present in the posterior of the dorsum

Parapodia

1. Parapodia are large flap like structures the may overlap and cover the dorsum
2. Parapodia arise from behind the head and extend along the length of the body occasionally folding to form open pockets
3. Parapodia are low and do not extend the full length of the body
4. Parapodia are irregularly edged, frilled, lobed or papillate

Gills

Gill location

1. Gills located halfway along body
2. Gills located toward the rear of the body
3. Gills located under the mantle skirt
4. Secondary gills are attached to the cerata
5. Secondary gills are attached to a mantle ridge

Gill arrangement

1. Gills in a circle
2. Gills in a part-circle
3. Gills form a goblet-like cup

Gill protection	
	1. Gills retract into a pocket in response to external stimuli
	2. Gills contract in response to external stimuli
	3. Gills are protected by anti-feedant substances secreted from glands on nearby structures such as a dorsal horn
Shell	
	1. Shell is ovate, globose with a flattened of depressed spire
	2. Shell is coiled and has spiral grooves
	3. Shell is coiled and has spiral bands
	4. Shell is thin, fragile and may be transparent
	5. Shell is a bivalve
	6. Shell is flattened or limpet like
	7. Shell is covered by a thin periostracum or is partially internal

An illustrated information page is available for each feature (Fig. 1) accessed by selecting a multimedia hyperlink which opens the page in a web-browser. These pages contain a feature definition, a list of feature state selection choices, images designed to augment the users' understanding and photo captions.

The key incorporates all extant families of epibenthic heterobranch sea slugs from the waters of the Australian Maritime Contiguous Zone, but not including Christmas Is., Cocos (Keeling) Is., Heard Is., Macquarie Is., Norfolk Is. and the Australian Antarctic Territory as defined by Geoscience Australia (2016). A total of 59 families and

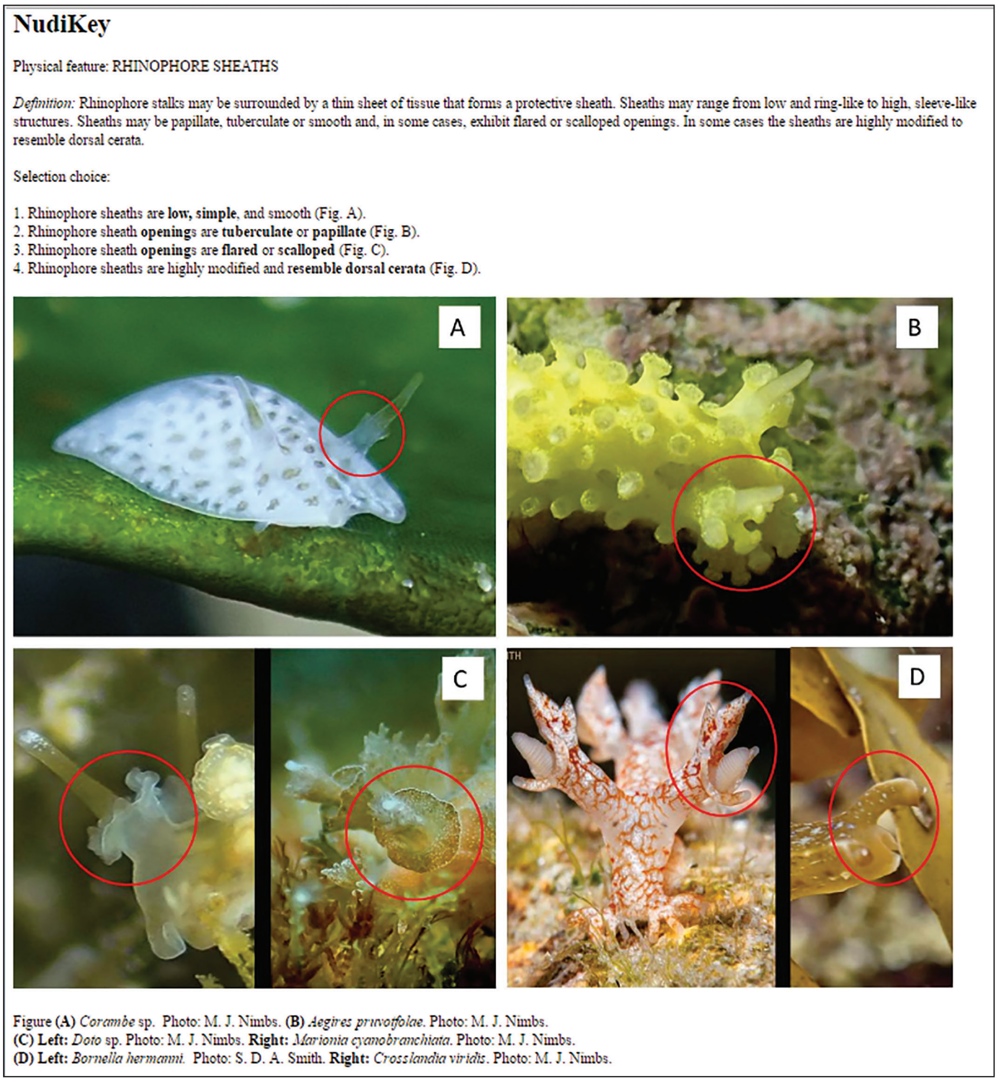


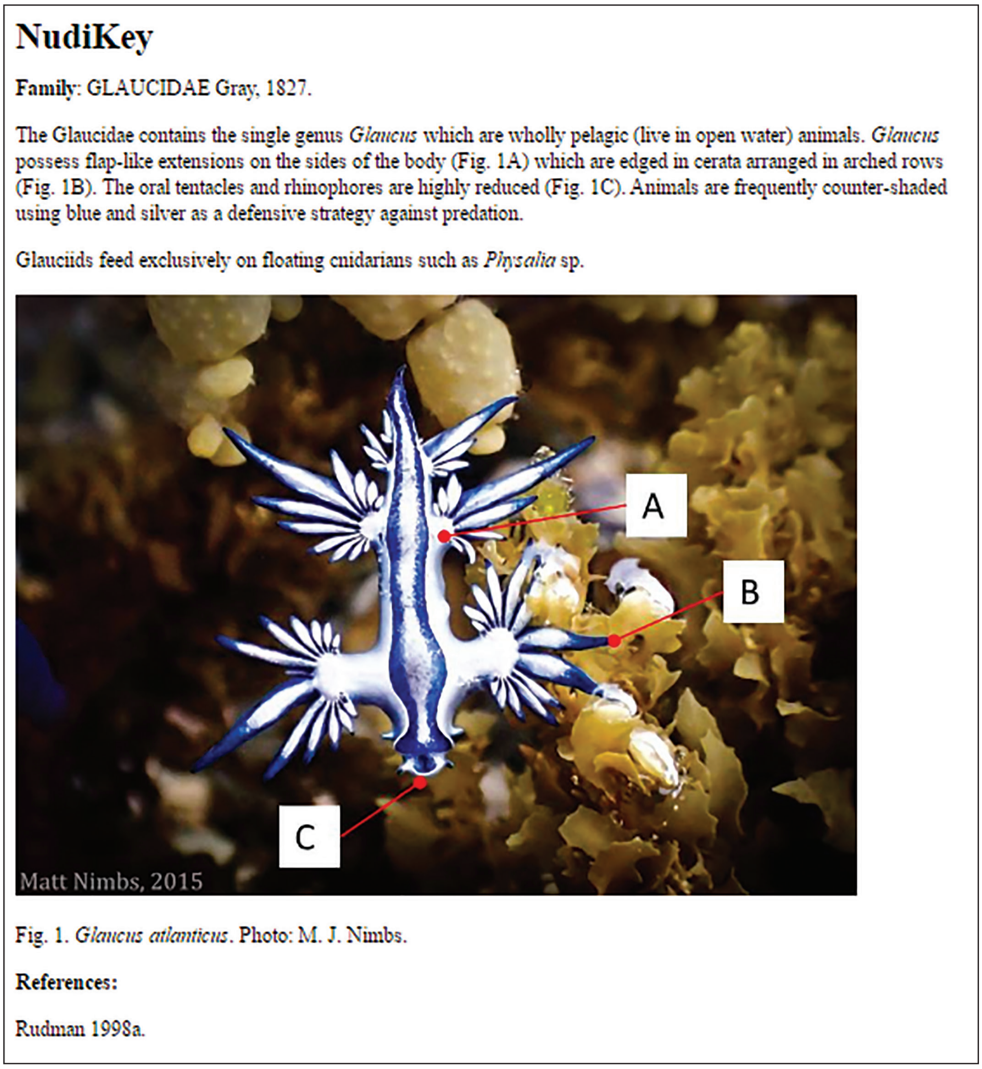
Figure 1. Screen shot of the feature information page for 'Rhinophore Sheaths'. Each information page is accessed from the key via a multimedia hyperlink and opens in a web-browser.

Table 2. List of Australian sea slug families included as identifiable entities in NudiKey.

Order	Family
Acteonoidea	
	Aplustridae
	Bullinidae
Anaspidea	
	Akeridae
	Aplysiidae
Cephalaspidea	
	Aglajidae
	Bullidae
	Gastropteridae
	Haminoeidae
	Philinidae
	Colpodaspididae
Nudibranchia	
	Actinocyclidae
	Aegiridae
	Aeolidiidae
	Arminidae
	Bornellidae
	Charcotiidae
	Chromodorididae
	Corambidae
	Dendrodorididae
	Discodorididae
	Dorididae
	Doridomorphidae
	Dotidae
	Embletoniidae
	Eubranchidae
	Facelinidae
	Fionidae
	Flabellinidae
	Glaucidae
	Goniodorididae
	Gymnodorididae
	Hancockiidae
	Hexabranchidae
	Lomanotidae
	Madrellidae
	Okadaiidae
	Onchidorididae
	Phyllidiidae
	Pinufiidae
	Polyceridae
	Kalinginae
	Nembrothinae
	Polycerinae
	Triophinae
	Proctonotidae
	Scyllaeidae
	Tergipedidae
	Tethydidae
	Tritoniidae
Pleurobranchomorpha	
	Pleurobranchidae
	Pleurobranchaeidae
Runcinacea	
	Runcinidae
	Ilbiidae
Sacoglossa	
	Boselliidae
	Caliphyllidae
	Costasiellidae
	Hermaeidae
	Juliidae
	Limapontiidae
	Oxynoidae
	Plakobranchidae
	Volvatellidae
Umbraculida	
	Tylodinidae
	Umbraculidae

5 subfamilies (Table 2) are listed as identifiable entities. These are arranged in a similar manner to features and states within the interface: each family is listed subordinate to its taxonomic order in a collapsible list. For each entity an information page which includes the family name, taxonomic authority, a brief description of diagnostic external morphological characters and distinctive behavioural traits (if relevant) is accessible via a multimedia hyperlink within the key and opens in a web-browser (Fig. 2). Where possible, photographs of living animals are included with important diagnostic characters labelled. In some cases, information on diet and spawn characteristics are also included as supplemental data. Information sources are cited under references with full bibliographic information available on the Acknowledgements, photo credits and references HTML page.

The presence of ontogenic variation among many sea slugs (Thompson 1976a) mean that juvenile specimens <10 mm crawl length may not exhibit consistent morphological characteristics to allow identification. Thus, the key is generally suitable only for identifying adult specimens. Notwithstanding, there are some families (e.g. Okadaiidae Thiele, 1931) that, at maturity, are <10 mm crawl length



NudiKey

Family: GLAUCIDAE Gray, 1827.

The Glaucidae contains the single genus *Glaucus* which are wholly pelagic (live in open water) animals. *Glaucus* possess flap-like extensions on the sides of the body (Fig. 1A) which are edged in cerata arranged in arched rows (Fig. 1B). The oral tentacles and rhinophores are highly reduced (Fig. 1C). Animals are frequently counter-shaded using blue and silver as a defensive strategy against predation.

Glauciids feed exclusively on floating cnidarians such as *Physalia* sp.

Fig. 1. *Glaucus atlanticus*. Photo: M. J. Nimbs.

References:

Rudman 1998a.

Figure 2. Screen shot of entity information page for the family Glaucidae. Each page is accessed from the key via a multimedia hyperlink and opens in a web-browser.

and consequently those families with diminutive taxa are accommodated in the key.

The Lucid score analyser tool was used to explore the efficiency of the key. Results are displayed in a histogram that illustrates the capacity of the feature states to distinguish between entity pairs (Fig. 3) The majority of entities have more than three (>3) unique differences and the average is ten. Although some entities have zero scores these comprise umbrella entities (the orders: Acteonoidea, Anaspidea, Cephalaspidea, etc) which were included merely to organise families into a taxonomically rational scheme within the entity hierarchy. Low scores, i.e where there are <3 differences between entities, are the product of those families that are difficult to separate from one another by external features alone such as the Flabellinidae and Facelinidae.

Identification procedure

The online user interface deploys via a Lucid 3 player available either through a Java applet or a web-based platform. The Java applet has greater flexibility, allowing frame resizing and enhanced functionality. Both options are available for launch from the NudiKey home page. The key interface displays a dynamic, user-friendly page comprising four frames: the top left frame contains the features which may be selected and the top right frame lists the entities from which the specimen may be identified. When a feature state is selected it will appear in the lower left frame under 'features chosen'. Selecting features progressively eliminates those entities for which the features do not apply from the list in the top right and discarded entities will appear in the lower right frame as 'entities discarded' (Figure 4).

As a multi-entry key, all features do not need to be 'answered' and the sequence in which features are selected does not affect the final outcome. To resolve an identification task, feature states are progressively chosen until only one entity remains in the top right frame or all states options are exhausted. In some cases it may not be possible to eliminate entities until only one remains, depending on the features that are available, particularly if identification is performed

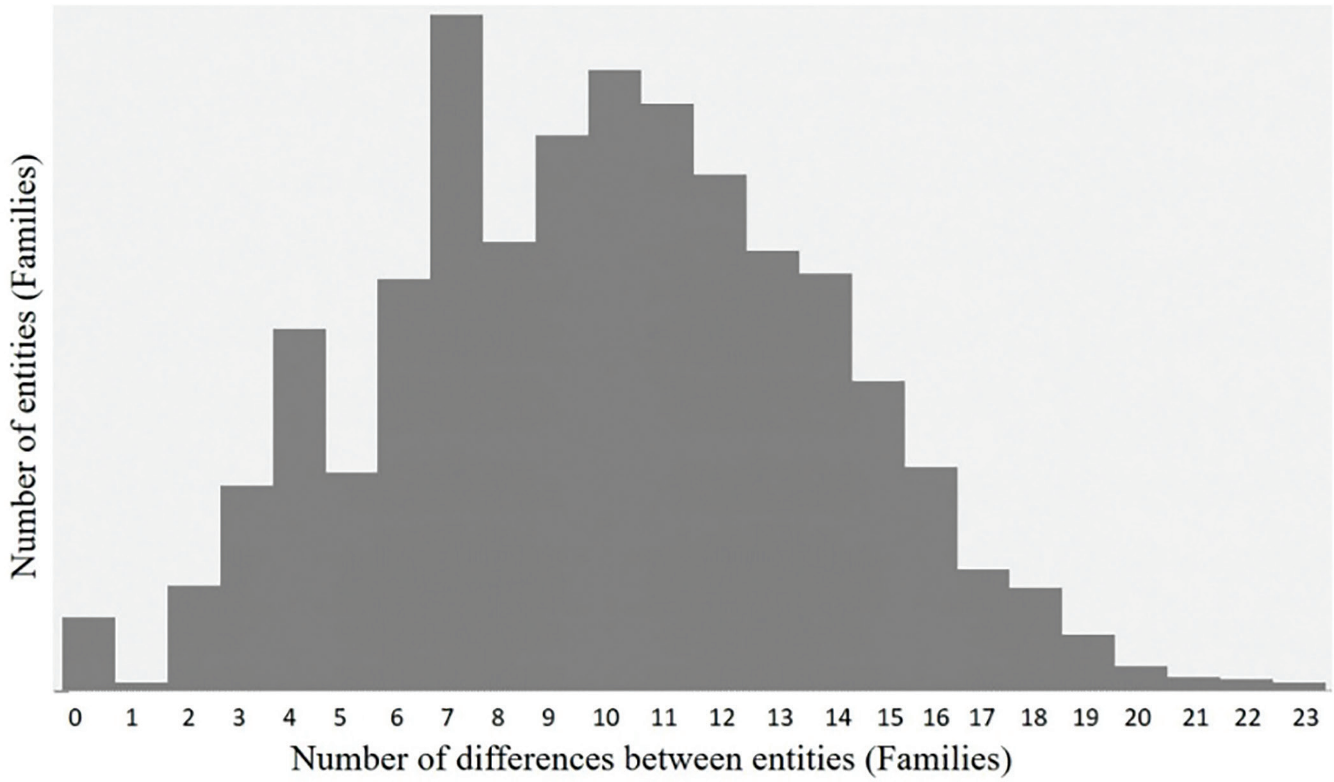


Figure 3. Analysis using the Lucid score analyser of the number of feature states (character states) that differentiate the entities (families).

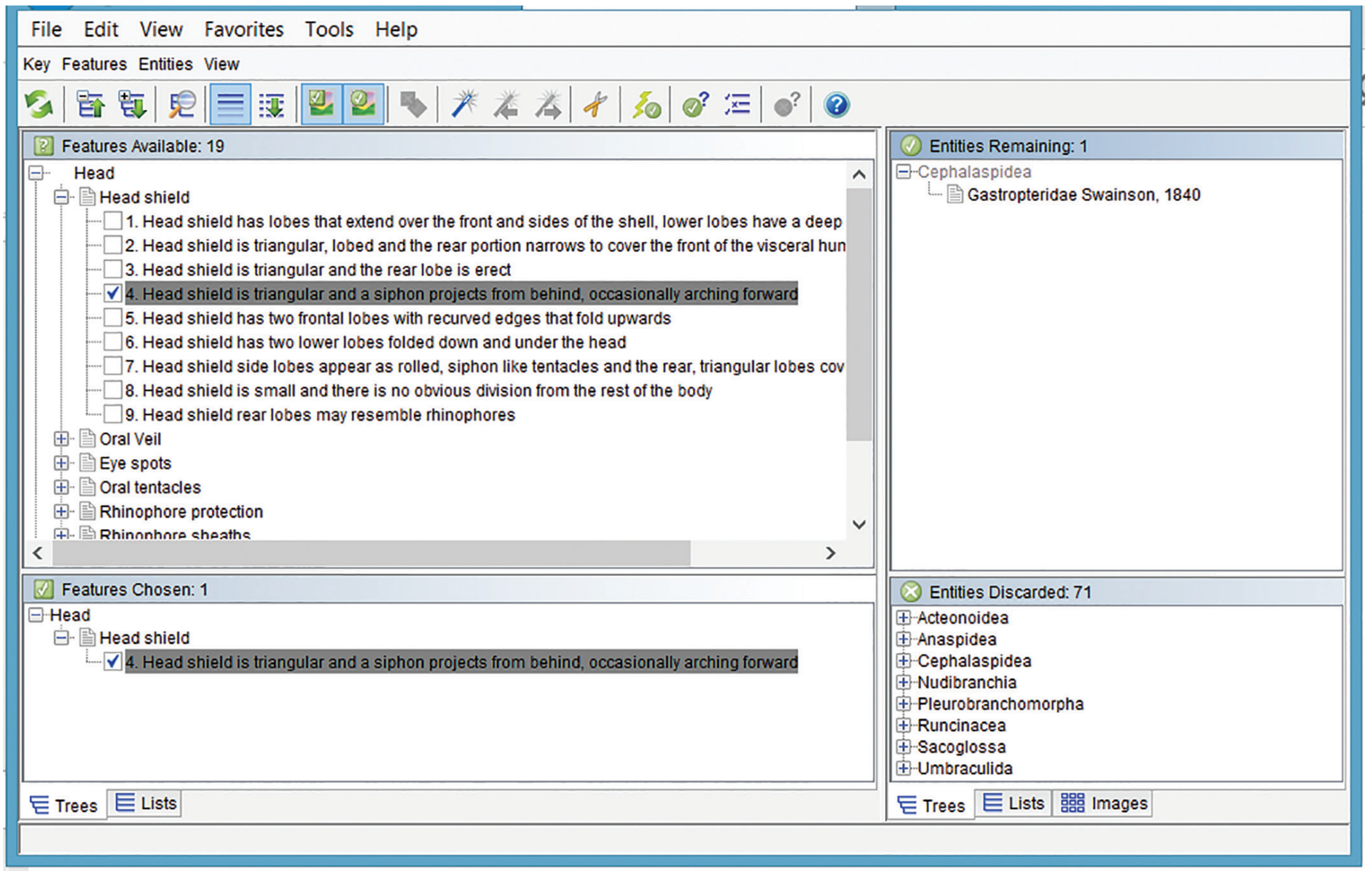


Figure 4. Screen shot of Lucid 3 illustrating the four frame interface: top left – 'Features Available' with 'head shield' feature and selectable states listed; bottom left – 'Features Chosen' shows the selected feature and state; top right – 'Entities Remaining' shows the only entity to which the selected feature and state apply; bottom right – 'Entities Discarded' shows all discarded entities.

using photographs only. Where multiple entities remain, reference to the information page for each remaining entities (Figure 3) may bring the user closer to an identification.

Several enhanced options are available when the key is accessed via the Java Applet. These includes *ranked* sorting, *filtered* sorting and *best* feature functions. In *ranked* mode, entities are assigned a percentage value as a measure of agreement with the selected feature states and arranged accordingly, with the highest ranked at the top of the list. In this mode entities are not eliminated. In *filtered* mode, entities that do not score a match with the selected feature state are eliminated from the 'entities remaining' list. Should multiple entities remain after the user has exhausted a particular specimen's character states the key may be switched between modes to explore the results offered by both. The *best* function guides the user to the most efficient feature within the key, based on its capacity to eliminate the greatest number of entities from those remaining. This tool may be used repeatedly to expedite the identification process.

Testing was carried out by nine independent volunteers ranging from experienced malacologists to uninitiated individuals. Numerous identification tasks, using photographic specimens, were undertaken with most resolved at first or second attempt. Feedback from testing resulted in various modifications, from small corrections to important alterations. Most importantly, testing revealed that identification of sea slugs to family using external morphological characters can be readily achieved and, in many cases, can be done using photographs alone, provided critical features are present. This reduces the need for destructive sampling or specimen collection.

Many species of sea slug remain undescribed and contemporary molecular studies continue to modify phylogenies. This state of flux is likely to render any traditional identification key obsolete within a relatively short period of time. Fortunately, online resources may be readily emended and updated with comparatively little effort, and the capacity to expand the key within the same web space aids in maintaining longevity, usefulness and accessibility. As a key to families, with the potential to eventually key to species level across all taxa, NudiKey is likely to remain relevant for some time.

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